PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group Art Unit 1808 :

In re application of :

HITOSHI NAGAOKA : INHIBITOR OF HEPATITIS B AND HIV ACTIVITY

Serial No. 08/519,293 :

Filed August 25, 1995 :

Examiner - I. Marx :

Pittsburgh, Pennsylvania

DECLARATION-2

Hon. Commissioner of Patents and Trademarks
Washington, D. C. 20231

Sir:

I, Hideo Sawadaishi, declare as follows:

1. I am a citizen of Japan, and residing at Sunny Homes Negishi B 101, Tsutsumidai 83, Noda-shi, Chiba-ken, Japan.

In March, 1975, I was graduated from a prefectural Shimizu Senior high school, Department of Industrial Chemistry.

Since April, 1975 till 1991, I have been an employee of Noda Shokkin Kogyou K.K., I was engaged in various research and development works in Lentinus edodes mycelium.

Since 1991, I have been an employee of NAGAOKA L.E.M. LABORATORY Co., LTD., and till the present time, I have been engaged in research and development works in various research and development works in Lentinus edodes mycelium.

2. I am familiar with the contents of the present invention as well

as of the prior art references cited in the official action.

3. In order to further support the unexpected results obtained from the extract of the present invention, I carried out the following Experiments I, II and III.

Experiment I

1 kg of solid culture medium (bagasse/rice bran : 9/1) was disentangled so that the amount of the bagasse fibers of 12-in mesh was 24 % by weight. To the disentangled solid culture medium were added 3.5 liters of purified water and 2 g of purified cellulase as an enzyme with keeping the solid culture medium at 37 ℃ for 1 hour, to give a bagasse-containing mixture.

Then, the mixture was further heated up to 70℃ and allowed to stand for 1 hour at the same temperature. By the heating, inactivation of the enzyme of the mixture was performed.

The resultant mixture was filtered through a filter cloth of about 100-mesh to obtain a filtrate. The thus obtained filtrate was subjected to lyophilization to obtain a powdery material (Sample 1).

With respect to Sample 1, the anti-Human Immunodeficiency Virus (HIV) effect was measured in the same manner as in Test Examples 1 to 10 of the Preparation Example 1. The results are shown in Table A.

Table A (Sample 1)

Test No.	Concentration (μ g/ml)	MT-4		MT-4/HIV	
		Absorbance	Viability (%)	Absorbance	Viability (%)
1	Control	1.058	100.0	0.093	8.8
2	3.9063	1.117	105.6	0.104	9.8
3	7.8125	1.130	106.8	0.108	10.2
4	15.6250	1.114	105.3	0.127	12.0
5	31.2500	1.138	107.6	0.134	12.7
6	62.5000	1.092	103.2	0.181	17.1
7	125.0000	0.964	91.1	0.266	25.1
8	250.0000	0.106	10.0	0.115	10.9
9	500.0000	0.026	2.5	0.030	2.8
10	1000.0000	0.023	2.2	0.026	2.5

Experiment II

A pharmaceutical Lentinus edodes mycelium extract for inhibiting human immunodeficiency virus activity (Sample 2) was prepared in the same manner as in Preparation Example 1 of the present specification except that the amount of the purified cellulase is changed to 0.5 g from 2.0 g.

With respect to Sample 2, the anti-Human Immunodeficiency Virus (HIV) effect was measured in the same manner as in Test Examples 1 to 10 of the Preparation Example 1. The results are shown in Table B.

Table B (Sample 2)

Test No.	Concentration (μg/ml)	MT-4		MT-4/HIV	
		Absorbance	Viability (%)	Absorbance	Viability (%)
1	Control	1.168	100.0	0.095	8.1
2	3.9063	1.242	106.3	0.182	15.6
3	7.8125	1.289	110.4	0.196	16.8
4	15.6250	1.308	112.0	0.331	28.3
5	31.2500	1.303	111.6	0.334	28.6
6	62.5000	1.292	110.6	0.494	42.3
7	125.0000	1.149	98.4	0.712	70.0
8	250.0000	0.127	10.9	0.165	14.1
9	500.0000	0.031	2.7	0.057	4.9
10	1000.0000	0.023	2.0	0.043	3.7

Experiment III

A pharmaceutical Lentinus edodes mycelium extract for inhibiting human immunodeficiency virus activity (Sample 3) was prepared in the same manner as in Preparation Example 1 of the present specification except that the amount of the purified cellulase is changed to 5 g from 2.0 g.

With respect to Sample 3, the anti-Human Immunodeficiency Virus (HIV) effect was measured in the same manner as in Test Examples 1 to 10 of the Preparation Example 1. The results are shown in Table C.

Table C (Sample 3)

Test No.	Concentration (μ g/ml)	MT-4		MT-4/HIV	
		Absorbance	Viability (%)	Absorbance	Viability (%)
1	Control	1.146	100.0	0.091	7.9
2	3.9063	1.229	107.2	0.167	14.6
3	7.8125	1.269	110.5	0.187	16.3
4	15.6250	1.305	113.9	0.324	28.3
5	31.2500	1.303	113.7	0.339	29.6
6	62.5000	1.298	113.3	0.496	43.3
7	125.0000	1.149	100.3	0.618	70.8
8	250.0000	0.117	10.2	0.158	13.8
9	500.0000	0.049	4.3	0.050	4.4
10	1000.0000	0.044	3.8	0.037	3.2

From the results shown in Tables A, B and C, and based on may knowledge, I conclude that:

The results of the Experiment I show that when the concentration is 125μ g/ml, the viability of the HIV-infected MT-4 cells to Sample 1 is as low as 25.1.

By contrast, the results of the Experiments I and II show that when the concentration is 125 μ g/ml, the viabilities of the HIV-infected MT-4 cells to Samples 1 and 2 are as high as 70.0 and 70.8, respectively.

Thus, according to the present invention, such high viability of the HIV-infected MT-4 cells can be attained.

4. I declare further that all statements made herein of my own knowledge are true and that statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

this 9th day of June, 1997

澤田石 英雄

Hideo SAWADAISHI